REMARKS

Claim 15 is amended merely to emphasize a pre-existing feature: that the probe is double-stranded, and that subsequent references to the same probe also refer to a double-stranded probe. This amendment introduces no new matter.

We disclose, and our claims are duly limited to a solid phase hybridization assay: an immobilized target hybridizes with a probe, wherein one of the two is double-stranded and the other is single-stranded. The single-stranded molecule is complementary to one of the strands of the double-stranded molecule, i.e. it has the complementary sequence and opposite orientation as one strand and the same sequence and orientation as the other strand of the double-stranded molecule (see p.5, lines 22-24). As explained below, this structural requirement is neither met nor suggested by the cited art. In fact, the invention is premised on and limited to a hitherto unexploited binding phenomenon, distinct from Hoogsteen binding of cited Bates reference, and the conventional single stranded - single stranded hybridization reactions of the Tyagi, Pease, Brown and Anderson references.

35USC102(b)

Claims 1, 2, 8, 11, 15, 16, 22 and 23

The structural requirements of our claims are neither met nor suggested by the cited Bates et al. (Nucleic Acids Res. 23, 3627-3632, 1995), which describes the entirely unrelated Hoogsteen triplex formation (p.3628, col.1, line 16). The three triplex-forming systems of Bates are shown in his Table 1: the cited reactions are (1)Bt-T30/A30/T30, (2)Bt-AY/AU/Pso-20 and (3)Bt-HD1/HD2/HD3. Figure 2 describes each of these molecules: in each case, you have the third single stranded molecule binding the backside of the purines of the immobilized double stranded molecule. Note that the third strands are always pyrimidine polymers (T30, Pso-20 or HD3; see Fig.2). There is no complementarity[2], as expressly required by our claims. Note that

[2] The Action appears to rely on an untenable construction of our claims, construing the requirement of "having complementarity" to mean merely having "a potential to be complementary". We believe the complementarity limitation of our claim is unconditional. We do not understand the Action's motivation for restating our claims, nor do we know the source of the proposed alternative language, nor do we understand what is intended or meant by "potential

even with Bates' polyT/polyA homopolymers, the orientation of the polyT probe binding is parallel (not complementary) with the polyA of the duplex (see Fig.2) and the polyA probe binding of the A30-A30-T30 triplex (p.3630) is antiparallel with the polyA - not with the polyT. Bates' solid phase Hoogsteen binding assay provides a useful system for studying the kinetics of this phenomenon, however it is inapplicable to other than pyrimidine probes and purine rich targets, and it is not a hybridization assay as claimed. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

35USC103(a)

Claims 1, 2, 8-13 and 15-23

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the

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to be complementary" - as a molecule either has complementarity or it does not. Perhaps the Action is inadvertently equating complementary with hybridized polynucleotides? The rejection seems more directed to a claim reciting conditional functional language like "capable of hybridizing with". In any event, we would be pleased to adopt in our claims any alternative language which might be preferred my the Examiner to emphasize that the required complementarity is not conditional.

single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Combining the two references would provide no more than the use of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described, or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claim 3.

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Anderson et al (1985 Nucleic Acid Hybridization: a practical approach p. 86-109). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The Anderson reference is cited for no more than the reuse of filters and probes after

hybridization. Combining the two references would provide no more than the reuse of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claims 14 and 24

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996), Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994) and Brown et al. (US Patent No. 5,807522). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Brown is cited for the use of poly-L-lysine to coat microarray substrates. Combining the three references would provide no more than the use of Tyagi's doubly labeled probes in a

conventional single strand - single strand hybridization reaction on a substrate coated with poly-L-lysine. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

The Examiner is invited to call the undersigned if he would like to amend the claims to clarify the foregoing or seeks further clarification of the claim language.

Applicants hereby petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any fees or credit any overcharges relating to this communication to our Deposit Account No. 19-0750 (order no. IN-0016-1).

Respectfully submitted,
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VERSION MARKED TO SHOW AMENDMENTS

15. (Amended) A method for immobilizing and detecting a polynucleotide probe, comprising the steps of:

contacting a polynucleotide target which is stably associated with a surface of a solid support, with a double-stranded polynucleotide probe [which is double-stranded] under conditions wherein the double-stranded probe hybridizes with the target; and

detecting specific hybridization of the double-stranded probe to the target.